IJAE
Vol. 118, n. 2: 189-203, 2013
ITALIAN JOURNAL OF ANATOMY AND EMBRYOLOGY

Review in Histology and Cell Biology

Current concepts in the treatment of cartilage damage. A review

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Submitted November 28, 2012; accepted revised March 28, 2013

Summary

A literature review of the treatment of cartilage defects was conducted, examining the current literature on the well-known treatments. In particular, advantages and drawbacks of each of the discussed treatments were evaluated considering outcomes available in literature. The literature search was conducted on PubMed and Scopus using appropriate keywords in relation to cartilage defects. Main research articles were selected for review.
Cartilage damage affects thousands of persons each year; they are treated with implants and surgery. A major problem in the treatment of cartilage defects is the inability of cartilage to repair, which reduces the effectiveness of the treatment. In addition, cyclic loading of joints further degrades cartilage even after treatment. In relation to the conditions of cartilage lesions and the features of patients, a specific treatment is required in each case. Current treatments are often unpredictable in results but result in long term improvements for many patients, especially young patients.
The well established treatments such as osteochondral implants, bone marrow stimulation techniques, chondrogenic cell implantations have advantages and drawbacks, so that the search has not been interrupted for new strategies, such as scaffold materials. In this review we describe benefits and disadvantages of the established methods of cartilage regeneration that seem to have a better long-term effectiveness.

Key words

Osteoarthritis; cartilage; tissue engeneering; scaffold; chondrocytes; mesenchymal stem cells.

Introduction

Hyaline cartilage covers the opposing osseous ends of every diarthrodial human joint (Pichler et al., 2013; Cardile et al., 2013). Many joints may be affected by osteoarthritis (OA), which represents a major health problem. In the knees, one of the protection mechanisms against cartilage wear comes from the presence of the two menisci (Loreto et al., 2012; Musumeci et al., 2012, 2013a, 2013b). To protect the underlying bone, articular cartilage allows a continuous and almost frictionless movement of the bony skeleton over years (Musumeci et al., 2011a, 2013c). Mature human chon-

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drocytes, located in lacunae within the matrix, represent only 5% to 10% of the total cartilage volume but are crucial to the maintenance of a stable extra-cellular matrix (ECM). Chondrocytes are responsible for anabolic and catabolic conversion of the ECM macromolecules (Loreto et al., 2011). The two main ECM components are type II collagen and large chondroitin sulfate proteoglycan aggregates. Type II collagen primarily endows the cartilage with its tensile strength, whereas aggrecan provides the osmotic resistance for cartilage to withstand compressive loads (Loreto et al., 2011). Without any neural, lymphatic or vascular supply, cartilage resists heavy mechanical load over years without degenerative changes. Due to its unique properties, cartilage shows little or no intrinsic capacity for an effective healing response. At most a fibrocartilaginous scar results in response to trauma. Major expression of type I collagen and too small amounts of aggrecan leads to a poor quality repair tissue that does not resist the common mechanical forces within the joint. The chondral defect results in a progressive degeneration and damage, which may lead to an early onset of osteoarthritis (Buckwalter, 2002; Musumeci et al., 2011b). The aim of any cartilage repair procedure is to restore the defect with an optimal repair tissue, mechanically stable, in order to prevent further degeneration.

Currently, there are three different clinical methods available for repairing a chondral defect: penetration of the subchondral bone, osteochondral plug transplantation and chondrocyte transplantation. None of the mentioned procedures is capable to generate hyaline cartilage and the clinical outcome needs to be further improved. Small sized articular lesions are commonly addressed arthroscopically by penetration of the underlying subchondral bone (Steadman et al., 2001) to promote a fibrous scar within the defect by invasion of adult mesenchymal stem cells (MSC). However, the reparative tissue does not withstand repetitive mechanical forces becsause of its poor quality, consisting mainly of collagen type I, and the clinical outcome deteriorates over time (Kreuz et al., 2006). Osteochondral plug transplantation, or ostechondral autograft transfer system (OATS), usually applied for mid-sized defects (Hangody and Fules, 2003), immediately recovers the joint surface. Pioneered by the work of Brittberg in 1994, cell-based treatment methods for repairing particularly large chondral lesions across the knee joint have been developed (Brittberg et al., 1994). Most articular cartilage tissue engineering approaches include the use of transplanted cells due to the low metabolic rate of native, mature chondrocytes. Traditionally, autologous articular chondrocytes are used (Brittberg et al., 1994), but allogenic chondrocytes (Dhollander et al., 2012), chondrocytes from other cartilaginous tissues, and chondroprogenitor cells have also been used (Klein-Nulend et al., 1998). Chondrocyte transplantation is one of the most successful techniques used to treat cartilage defects in humans (Peterson et al., 2003), though implanted grafts do not provide primary mechanical stability and various side effects, leading to procedure failure, have been reported (Knutsen et al., 2004; Wood et al., 2006). In this review we describe benefits and disadvantages of the established methods of cartilage regeneration that seem to have a better long-term effectiveness.

Osteochondral implants

Osteochondral implants are procedures of autologous osteochondral transplantation that involve transplantation of small cylindrical autologous osteochondral grafts

harvested from the articular surface and transferred to create a resurfaced area in the lesion. This technique offers some advantages including transplantation of hyaline cartilage and a brief rehabilitation period, furthermore the procedure requires only a single intervention. The limitations of this technique include donor site morbidity, a limited availability of grafts that can be harvested, the absence of fill and the potential dead space between grafts that may limit the quality of the repair. Site healing results in filling of the lesion with cancellous bone and a fibrocartilage-like cap. It seems that large grafts determine greater morbidity and therefore it is suggested to use small grafts and fill the lesion with biocompatible material that may help to prevent morbidity (Bedi et al., 2010). Hydroxyapatite, carbon fiber, polyglyconate B, compressed collagen, and polycaprolactones have been used in a canine model to estimate healing of site lesions (Feczko et al., 2003). All these biocompatible materials show a good integration with the surrounding cancellous bone, but compressed collagen shows the most favorable fibrocartilage covering. However, osteochondral implants are controversial procedures in relation to the possible and uncertain effects of high nanoparticle concentrations of biocompatible materials in the bloodstream. Despite this, the outcomes of autologous osteochondral transplantation are encouraging. Several authors have evaluated the technique performed for femoral, tibial, patellar chondral lesions and for osteochondritis dissecans lesions; they have reported up to 92% rate of good-to-excellent results after a period of follow-up of several months, a 3% rate of donor site morbidity and the magnetic resonance imaging performed at the time of final follow-up has revealed nearly complete fill in all plugs (Hangody and Fules, 2003; Ozturk et al., 2006; Miniaci and Tytherleigh-Strong, 2007; Hangody et al., 2008; Nho et al., 2008; Widuchowski et al., 2008).

An alternative technique of osteochondral implants is the osteochondral allograft transplantation. It involves graft transplantation of intact articular cartilage from cadaver into the lesion. Advantages of this procedure are to achieve precise surface architecture, immediate transplantation of hyaline cartilage as a single-stage procedure, the potential to replace large lesions, and no donor site morbidity (Bedi et al., 2010). Limitations are limited graft availability, high cost, risk of immunological rejection, possible incomplete graft incorporation, potential for disease transmission, and the technically demanding aspects of machining and sizing of the allograft (Bedi et al., 2010). In osteochondral allograft transplantation it is possible to use three kinds of grafts: fresh allografts, cryopreserved allografts, fresh-frozen allografts. Fresh osteochondral allografts are preferred because both freezing and cryopreservation decrease chondrocyte viability. Chondrocyte function maintains the dynamic homeostasis of the extracellular matrix and it is important to ensure long-term allograft survival in vivo (Bakay et al., 1998). Fresh osteochondral allografts, stored in Ringer solution at 4°C, are transplanted within one week. The rate of chondrocyte viability upon allograft storage in culture medium is higher (91%) than upon storage in lactate-Ringer solution (80%; Ball et al., 2004). The matrix properties and chondrocyte viability of stored fresh osteochondral allografts have been evaluated and although the biomechanical properties and matrix integrity of hyaline cartilage are preserved for up to twenty-eight days, the number of viable chondrocytes has been shown to decrease progressively over that time (Pearsall et al., 2004; Allen et al., 2005). Hyaline cartilage is an immune-privileged tissue because of its avascular matrix that preserves chondrocytes from the host immune reaction. The allograft bone is necrotic but it provides

a structural scaffold to support the articular surface during this gradual incorporation. Cryopreservation of allografts involves freezing of grafts in a nutrient-rich medium with cryoprotectants such as glycerol or dimethyl sulfoxide to minimize cellular freezing and maintain cell viability. A 77% rate of chondrocyte viability at one year has been reported for cryopreserved osteochondral allografts implanted into load-bearing sites in an animal model (Gole et al., 2004). Another study has demonstrated, at five years, degenerative changes at the articular surface of cryopreserved allografts compared with fresh allografts (Bakay et al., 1998). Cryopreserved allografts achieve better results compared with fresh allografts (Schachar et al., 1999). Fresh-frozen preservation of allografts has the advantages of reduced immunogenicity and decreased disease transmission but is hampered by lower chondrocyte viability. The process of deep-freezing to 280°C destroys the viability of articular cartilage cells within the grafts and studies have demonstrated deterioration of cells and matrix (Gole et al., 2004). Several studies have been performed to assess the outcome of osteochondral allograft transplantation and they report good-to-excellent results in up to 86% cases even after many years from transplantation (Bugbee and Convery, 1999; Chu et al., 1999; Davidson et al., 2007). Overall, the best results with allograft transplantation have been reported for the treatment of osteochondritis dissecans lesions of the knee (Emmerson et al., 2007). The outcome is less reliable and predictable in primary osteoarthritis, inflammatory arthropathy, limb malalignment, bipolar lesions of the knee and patella-femoral chondral lesions (Jamali et al., 2005).

Bone Marrow Stimulation

Bone marrow stimulation techniques are the most widely used methods for the treatment of symptomatic small lesions of articular cartilage. Microfracture surgery is performed according to the method described by Steadman (Steadman et al., 2001). This technique uses an awl to perform microfractures into the intracortical bone. Neighboring mesenchymal stem cells leak into these holes and form a combination of cartilage and fibrous tissue with varying amounts of type-II collagen content (Frisbie et al., 2003; Steadman et al., 2003; Knutsen et al., 2004; Gobbi et al., 2005). The total concentration of mesenchymal stem cells is rather low and decreases with age (Tran-Khanh et al., 2005). The formation of a stable blood clot that fills the lesion is important, and it has been correlated with the success of the microfracture surgery technique, therefore unstable clots that are only partially adherent or fill only a portion of the lesion will repair in a suboptimal manner (Frisbie et al., 2003). The creation of a restricted lesion bordered by healthy cartilage is essential for obtaining an optimal filling of the lesion with a clot and the adhesion of this one (Mithoefer et al., 2006; Asik et al., 2008). The layer of calcified cartilage underlying the lesion must be removed to ensure the adhesion of the clot (Frisbie et al., 2006). The postoperative scheme is important for an optimal result of the microfracture surgery (Steadman et al., 2007), it consists of a continuous passive motion, 6-8 hours a day, for six weeks (Steadman et al., 2003; Gill et al., 2006). Data show that microscopic examination of the mandibular condylar cartilage of the animals placed on continuous passive motion showed a marked increase in thickness compared with the condylar cartilage found on the control animals, because of stimulation of the mesenchymal stem cells

to differentiate (Mussa et al., 1999). Unlimited movement is normally allowed after two months and a return to full activity is allowed after three months (Steadman et al., 2003; Gill et al., 2006).

The overall clinical results with microfracture surgery show improved articular function in 70% to 95% of patients, and most of the improvement has been shown in the first two postoperative years while deterioration of articular has been detected function after two years; the reason for this deterioration has not yet been identified (Steadman et al., 2003; Knutsen et al., 2004; Gobbi et al., 2005). Some studies provide clinical evidence that repair cartilage volume plays a critical role in the durability of functional improvement in the knee after microfracture surgery. Results of these studies highlight that there are patients who do not form a sufficient amount of repair cartilage after microfracture surgery and they have only a temporary functional improvement, in contrast with patients with a high fill volume that have superior functional results and durability (Mithoefer et al., 2005). Data indicate that deterioration of knee function is not limited to patients with a poor fill grade and that other factors should be considered such as age and body-mass index. Some authors show that a lower body-mass index is correlated with better results; it seems that an excessive body-mass index is a contraindication for microfracture surgery in the knee (Mithoefer et al., 2005; Mithoefer et al., 2006). Other authors argue that the age is an independent predictor of functional improvement; their studies show that articular cartilage repair after microfracture surgery in patients who are less than thirty years old have better clinical outcomes (Steadman et al., 2003; Knutsen et al., 2004). A better functional result in patients younger than thirty years may be attributed to an age-dependent qualitative and quantitative difference in metabolic activity in the repaired cartilage (Martin and Buckwalter, 2003). Cartilage lesions untreated for prolonged periods may lead to the development of early degenerative joint changes, particularly at the margin of the lesions, explaining the worse results with late repair observed in some investigations. Some authors emphasize the importance of early surgical treatment of articular cartilage lesions (Mithoefer et al., 2005). In summary, microfracture surgery provides subjective functional improvement and significantly increased activity levels in patients with isolated articular cartilage lesions. The best functional results are observed in patients with a good volume of repair cartilage, a lower body-mass index and a shorter preoperative duration of symptoms. The drawback of this procedure is the high probability of recurrence of symptoms after one to two years (Negrin et al., 2012).

Procedures to improve the stability of the clot and the filling of the lesion have been described. In an ovine model, stabilization of the blood clot by the addition of chitosan, an adhesive and thrombogenic polymer, resulted in a better filling of the lesion and tissue healing after microfracture surgery (Hoemann et al., 2005). Growth factors have been used for cartilage repair *in vivo*, so insulin-like growth factor has improved both quantity and quality of cartilage tissue repair and has reduced the severity of postoperative inflammation in an equine model (Nixon et al., 2005). Platelet-derived growth factor is a potent mitogen for mesenchymal cells; some studies have shown promising results in relation to its ability to stimulate the formation of hyaline cartilage and the proliferation of chondrocytes (Akeda et al., 2006; Mishra et al., 2009). Finally, Strauss et al. evaluated the results of microfracture surgery with or without hyaluronic acid supplement in a New Zealand white rabbit model (Strauss et

al., 2009). After three months from microfracture surgery, the histological examination of tissue repair has revealed better filling of the lesion and less degenerative changes than in controls.

However, none of these adjuvants to microfracture surgery has yet been tested on humans, and therefore their clinical efficacy has yet to be tested.

Chondrogeneic cell implantation

Autologous chondrocyte implantation (ACI: Fig. 1) is a procedure that has the aim of repairing chondral defects by implanting cartilage cells. There are three generations of ACI:

1st generation, with a chondrocyte suspension implanted under a periosteal flap;

2nd generation, with a chondrocyte suspension implanted under a collagen membrane;

3rd generation, with cells grown on or in matrices implanted as immature grafts into the defects.

The benefit of ACI is the development of hyaline-like cartilage rather than fibrocartilage in the lesion, leading to better long-term outcomes. This procedure requires that cartilage cells are removed from the injured knee and grown in culture. The cells are then implanted into the defect where they grow and fill the lesion regenerating a

Fig. 1

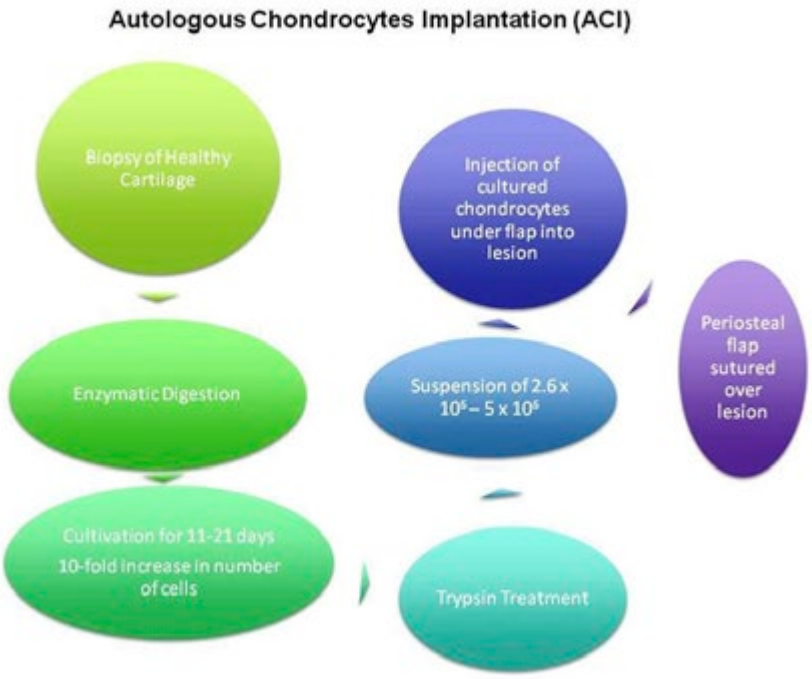

Figure 1 – Diagram of autologous chondrocytes transplantation (ACI).

cartilage surface (Bedi et al., 2010). Studies on ACI in patients monitored for a long period have reported good-to-excellent results based on pain and quality of life, and biopsies have shown that the regenerated tissue had the appearance of hyaline cartilage (Brittberg et al., 1994; Zaslav et al., 2009). Autologous chondrocyte implantation has some limitations. The drawbacks of this method are the requirement for two surgical interventions, a very slow recovery (12 to 18 months) and a high cost (Brittberg et al., 1994). Complications have also been reported (Wood et al., 2006; Bedi et al., 2010) as well as adverse events such as periosteal hypertrophy and graft failure which may require further operation.

In matrix-associated chondrocyte implantation the cells are included into a porcine type I/III collagen membrane. This membrane has large interstices between fibers, where chondrocytes settle and proliferate. This procedure minimizes donor site morbidity, prevents dedifferentiation of chondrocytes during culture and may be performed at arthroscopy (Bedi et al., 2010). Using fibrin glue as matrix, a completely attached graft has been observed in 88% patients (Marlovits et al., 2005). The efficacy of matrix-associated chondrocyte implantation has been also evaluated in an ovine model; magnetic resonance imaging has shown that matrix associated chondrocyte implantation is superior to lack of treatment (Dorotka et al., 2005; Jones et al., 2008). Zheng et al. (2007) have analysed chondrocytes seeded on a type I/III collagen scaffold: the cells appeared spherical and integrated into the matrix and expressed type II collagen and aggrecan; parallel histological analyses on patients treated by implantation of such cell-bearing scaffolds showed 75% hyaline-like cartilage regeneration after six months. Similar studies on patients with a longer follow-up found that the repair process progressed to total defect filling with complete integration (Smith et al., 2005; Trattnig et al., 2005; Behrens et al., 2006; Wright, 2009). Another procedure takes advantage of a "sandwich" technique, by which two matrix membranes are cut to the size of the defect and one of them is fixed with fibrin glue to the base of the defect itself while the second one is implanted on top of the other and is sealed with fibrin glue to the adjacent cartilage. This technique is suitable for deep osteochondral defects and is combined with bone filling at the bottom of the defect. In one report, all patients had improved function within six months (Bartlett et al., 2005).

Scaffolds analogous to the natural three-dimensional extracellular matrix may provide important microenvironmental clues to cells. A wide array of materials has been used in various *in vitro* and *in vivo* studies for articular cartilage engineering. Candidate materials must be biocompatible and accommodate cell adhesion, proliferation, and matrix synthesis. Scaffolds that are most often studied in cartilage tissue engineering include hydrogels made from collagen (Dorotka et al., 2005), fibrin (Peretti et al., 2000; Ameer et al., 2002), agarose, and synthetic peptides (Kisiday et al., 2002; Kisiday et al., 2004); sponge-like scaffolds manufactured from materials such as collagen, polyglycolic acid, polylactic acid (Grande et al., 1997), and polyurethane (Grad et al., 2005; Gogolewski et al., 2008); materials with a naturally-occurring porous structure, such as coral, devitalized articular cartilage (Hangody et al., 2008) and hyaluronan-based scaffolds (Kang et al., 2009). Hyaluronan-based scaffolds provide the autologous chondrocytes a scaffold of hyaluronan derivatives. This is a procedure for cartilage repairing that employs a biodegradable, three-dimensional scaffold for cell proliferation. A benzylic ester of hyaluronic acid is used to generate a scaffold with variably sized interstices between 20 mm thick fibers. The three-dimensional scaffold provides

the structural support for cell contact and matrix deposition prevents dedifferentiation of autologous chondrocytes even after long periods and promotes the expression of chondrocyte-specific markers (Filova et al., 2008). Advantages of this procedure are a more uniform cell distribution, avoidance of periosteal harvest and implantation, and increased technical ease without the need for suturing to adjacent articular cartilage. Histological analysis of implantation of hyaluronan-based scaffolds seeded with autologous chondrocytes shows hyaline-like cartilage in the lesion as soon as twelve months after implantation. Marcacci et al. (2005) have reported the outcome of hyaluronan-based scaffold in patients with a chondral defect; after a period of thirty-eight months following treatment, 92% patients had improved their function and arthroscopic evaluation revealed complete coverage of the defect with a hyaline-like reparative tissue. In another study the hyaluronan-based scaffolds seeded with autologous chondrocytes has been evaluated as a treatment for patello-femoral chondral lesions; magnetic resonance imaging at twenty-four months has revealed that 71% cases has complete filling and absence of subchondral edema, with a positive correlation with clinical outcome; arthroscopy revealed complete filling of the defects with reparative tissue that was characterized as hyaline-like histologically (Gobbi et al., 2006). Nowadays the techniques of tissue engineering have progressed further and, for example, they can provide collagen matrices seeded with chondrocytes. Autologous chondrocytes are harvested in a manner analogous to conventional autologous chondrocyte implantation. The cells are then included in a type-I bovine collagen matrix and cultured ex vivo. The cell-scaffold construct is subjected to mechanical stimulation with use of hydrostatic pressure, because the application of mechanical load stimulates chondrocytes to produce increased amounts of type-II collagen, aggrecan, and other components of the hyaline extracellular matrix (Bedi et al., 2010). The cell-scaffold construct is then fixed to the patient with a collagen bioadhesive, which is applied to the base of the defect (Ryan et al., 2009). The clinical outcome associated to date with tissue-engineered collagen matrices seeded with autologous chondrocytes show good long-term results in several cases of matrix associated ACI.

Crawford et al. (2009) reported the results of a clinical trial on eight patients with twenty-four months of follow-up. Magnetic resonance imaging showed that seven of the eight patients had complete or nearly complete filling of the defect at one year, and six of the eight maintained good filling at two years.

In procedures of cartilage regeneration that require an expansion of stem cells or chondrocytes, various growth factors, i.e. fibroblast growth factor-2, transforming growth factor-β (TGF- β), insulin-like growth factor-1 and osteogenic protein-1, have been used to modulate chondrocyte phenotype, proliferation and biosynthetic activity. In particular, chondrogenic medium containing dexamethasone and TGF-β1 has been developed to induce chondrogenic differentiation from chondroprogenitor cells (Johnstone et al., 1998). However, when considering the natural *in vivo* repair environment, it must be also considered what would be the natural source of these factors. Li et al. (2009) demonstrated that chondrogenesis can be induced *in vitro* in absence of TGF-β when mechanical load is applied. Under these conditions the cells up-regulate the synthesis of TGF-β and this is responsible for the chondrogenic response. Thus, in the natural *in vivo* environment a suitable rehabilitation protocol would be required to increase the synthesis of TGF-β leading to appropriate chondrogenic response.

Discussion

Cartilage damage due to deterioration or autoimmune disorders is generally chronic because of the low regenerative ability of cartilage tissue. One of the first, but more invasive, clinical cartilage treatments is the joint replacement. This is a procedure of orthopedic surgery in which the arthritic or dysfunctional joint surface is replaced with an orthopedic prosthesis. In this treatment, a highly cross-linked polymer cap connected to metal components is used to replace the function of the natural cartilage. However joint replacements have a lifetime of 15-20 years (van Ooij et al., 2003), and this may be a problem for younger people. This treatment involves substantial postoperative pain, and it is necessary a vigorous physical rehabilitation. The recovery period may be 6 weeks or longer and may involve the use of mobility aids. Joint replacement is considered as a treatment when severe joint pain or dysfunctions are not alleviated by less-invasive therapies. Because of these reasons, tissue engineering of natural cartilage tissue has become an attractive new area of research. In this review, we have described the most widely used techniques in the treatment of cartilage lesions to solve the problem of the management of cartilage defects.

Conclusions

In conclusion, the treatment of articular cartilage defects can be approached by different procedures in relation to cartilage lesions. Current data suggest that favorable outcomes of microfracture surgery and whole-tissue transplantation of allografts or autografts are achieved for the treatment of cartilage defects. Further in vivo and in vitro studies must be carried out in order to confirm their successful clinical outcomes.

Acknowledgments

The authors would like to thank Prof. Mauro Alini from the AO Research Institute, Davos Platz, Switzerland, Prof. Wenberg Annelie Martina, Medical University of Graz, Austria, Prof. Jennifer Elisseeff, Department of Biomedical Engineering and Orthopedic Surgery, Johns Hopkins University of Baltimore, Meryland, USA, Prof. M.L Carnazza, Department of Bio-Medical Science, Section of Human Anatomy and Histology, University of Catania for her valuable help.

References

Akeda K., An H., Okuma M., Attawia M., Miyamoto K., Thonar E., Lenz M., Sah R., Masuda K. (2006) Platelet-rich plasma stimulates porcine articular chondrocyte proliferation and matrix biosynthesis. Osteoarthr. Cartil. 14: 1272-1280.

Allen R.T., Robertson C.M., Pennock A.T., Bugbee W.D., Harwood F.L., Wong V.W., Chen A.C., Sah R.L., Amiel D. (2005) Analysis of stored osteochondral allografts at the time of surgical implantation. Am. J. Sports Med. 33: 1479-1484.

Ameer G.A., Mahmood T.A., Langer R. (2002) A biodegradable composite scaffold for cell transplantation. J. Orthop. Res. 20: 16-19.

Asik M., Ciftci F., Sen C., Erdil M., Atalar A. (2008) The microfracture technique for the treatment of full-thickness articular cartilage lesions of the knee: midterm results. Arthroscopy 24: 1214-1220.

Bakay A., Csonge L., Papp G., Fekete L. (1998) Osteochondral resurfacing of the knee joint with allograft. Clinical analysis of 33 cases. Int. Orthop. 22(5): 277-281.

Ball S.T., Amiel D., Williams S.K., Tontz W., Chen A.C., Sah R.L., Bugbee W.D. (2004) The effects of storage on fresh human osteochondral allografts. Clin. Orthop. Relat. Res. 418: 246-252.

Bartlett W., Skinner J.A., Gooding C.R., Carrington R.W.J., Flanagan A.M., Briggs T.W.R., Bentley G. (2005) Autologous chondrocyte implantation versus matrix-induced autologous chondrocyte implantation for osteochondral defects of the knee. J. Bone Joint Surg. Br. 87B: 640-645.

Bedi A., Feeley B.T., Williams R.J. (2010) Management of articular cartilage defects of the knee. J. Bone Joint Surg. Am. 92A: 994-1009.

Behrens P., Bitter T., Kurz B., Russlies M. (2006) Matrix-associated autologous chondrocyte transplantation/implantation (MACT/MACI) - 5-year follow-up. Knee 13: 194-202.

Brittberg M., Lindahl A., Nilsson A., Ohlsson C., Isaksson O., Peterson L. (1994) Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. N. Engl. J. Med. 331: 889-895.

Buckwalter J. (2002) Articular cartilage injuries. Clin. Orthop. Relat. Res. 402: 21-37.

Bugbee W.D., Convery F.R. (1999) Osteochondral allograft transplantation. Clin. Sports Med. 18: 67-75.

Cardile V., Musumeci G., Sicurezza E., Caggia S., Rusu M.C., Leonardi R., Loreto C. (2013) TRAIL and its receptors DR5 and DcR2 expression, in orthodontic tooth movement. Histol Histopathol. 28: 933-940.

Chu C.R., Convery F.R., Akeson W.H., Meyers M., Amiel D. (1999) Articular cartilage transplantation - Clinical results in the knee. Clin. Orthop. Relat. Res. 360: 159-168.

Crawford D.C., Heveran C.M., Cannon W.D., Foo L.F., Potter H.G. (2009) An autologous cartilage tissue implant NeoCart for treatment of grade III chondral injury to the distal femur. Prospective clinical safety trial at 2 years. Am. J. Sports Med. 37: 1334-1343.

Davidson P.A., Rivenburgh D.W., Dawson P.E., Rozin R. (2007) Clinical, histologic, and radiographic outcomes of distal femoral resurfacing with hypothermically stored Osteoarticular allografts. Am. J. Sports Med. 35: 1082-1090.

Dhollander A.A., Verdonk P.C., Lambrecht S., Verdonk R., Elewaut D., Verbruggen G., Almqvist K.F. (2012) Midterm results of the treatment of cartilage defects in the knee using alginate beads containing human mature allogenic chondrocytes. Am. J. Sports Med. 40: 75-82.

Dorotka R., Windberger U., Macfelda K., Bindreiter U., Toma C., Nehrer S. (2005) Repair of articular cartilage defects treated by microfracture and a three-dimensional collagen matrix. Biomaterials 26: 3617-3629.

Emmerson B.C., Gortz S., Jamali A.A., Chung C., Amiel D., Bugbee W.D. (2007) Fresh osteochondral allografting in the treatment of osteochondritis dissecans of the femoral condyle. Am. J. Sports Med. 35: 907-914.

Feczko P., Hangody L., Varga J., Bartha L., Dioszegi Z., Bodo G., Kendik Z., Modis L. (2003) Experimental results of donor site filling for autologous osteochondral mosaicplasty. Arthroscopy 19: 755-761.

Filova E., Jelinek F., Handl M., Lytvynets A., Rampichova M., Varga F., Cinatl J., Soukup T., Trc T., Amler E. (2008) Novel composite hyaluronan/type I collagen/fibrin scaffold enhances repair of osteochondral defect in rabbit knee. J. Biomed. Mater. Res. B Appl. Biomater. 87B: 415-424.

Frisbie D.D., Morisset S., Ho C.P., Rodkey W.G., Steadman J.R., McLlwraith C.W. (2006) Effects of calcified cartilage on healing of chondral defects treated with microfracture in horses. Am. J. Sports Med. 34: 1824-1831.

Frisbie D.D., Oxford J.T., Southwood L., Trotter G.W., Rodkey W.G., Steadman J.R., Goodnight J.L., McIlwraith C.W. (2003) Early events in cartilage repair after subchondral bone microfracture. Clin. Orthop. Relat. Res. 407: 215-227.

Gill T.J., Asnis P.D., Berkson E.M. (2006) The treatment of articular cartilage defects using the microfracture technique. J. Orthop. Sports Phys. Ther. 36: 728-738.

Gobbi A., Kon E., Berruto M., Francisco R., Filardo G., Marcacci M. (2006) Patellofemoral full-thickness chondral defects treated with hyalograft-C. A clinical, arthroscopic, and histologic review. Am. J. Sports Med. 34: 1763-1773.

Gobbi A., Nunag P., Malinowski K. (2005) Treatment of full thickness chondral lesions of the knee with microfracture in a group of athletes. Knee Surg. Sports Traumatol. Arthrosc. 13: 213-221.

Gogolewski S., Gorna K., Zaczynska E., Czary A. (2008) Structure-property relations and cytotoxicity of isosorbide-based biodegradable polyurethane scaffolds for tissue repair and regeneration. J. Biomed. Mater. Res. A 85A: 456-465.

Gole M.D., Poulsen D., Marzo J.M., Ko S.H., Ziv I. (2004) Chondrocyte viability in press-fit cryopreserved osteochondral allografts. J. Orthop. Res. 22: 781-787.

Grad S., Lee C.R., Gorna K., Gogolewski S., Wimmer M.A., Alini M. (2005) Surface motion upregulates superficial zone protein and hyaluronan production in chondrocyte-seeded three-dimensional scaffolds. Tissue Eng. 11: 249-256.

Grande D.A., Halberstadt C., Naughton G., Schwartz R., Manji R. (1997) Evaluation of matrix scaffolds for tissue engineering of articular cartilage grafts. J. Biomed. Mater. Res. 34: 211-220.

Hangody L., Fules P. (2003) Autologous osteochondral mosaicplasty for the treatment of full-thickness defects of weight-bearing joints - Ten years of experimental and clinical experience. J. Bone Joint Surg. Am. 85A: 25-32.

Hangody L., Vasarhelyi G., Hangody L.R., Sukosd Z., Tibay G., Bartha L., Bodo G. (2008) Autologous osteochondral grafting-technique and long-term results. Injury 39: S32-S39.

Hoemann C.D., Hurtig M., Rossomacha E., Sun J., Chevrier A., Shive M.S., Buschmann M.D. (2005) Chitosan-glycerol phosphate/blood implants improve hyaline cartilage repair in ovine microfracture defects. J. Bone Joint Surg. Am. 87A: 2671-2686.

Jamali A.A., Emmerson B.C., Chung C., Convery F.R., Bugbee W.A. (2005) Fresh osteochondral allografts - Results in the patellofemoral joint. Clin. Orthop. Relat. Res. 437: 176-185.

Johnstone B., Hering T.M., Caplan A.I., Goldberg V.M., Yoo J.U. (1998) In vitro chondrogenesis of bone marrow-derived mesenchymal progenitor cells. Exp. Cell Res. 238: 265-272.

Jones C.W., Willers C., Keogh A., Smolinski D., Fick D., Yates P.J., Kirk T.B., Zheng M.H. (2008) Matrix-induced autologous chondrocyte implantation in sheep. Objective assessments including confocal arthroscopy. J. Orthop. Res. 26: 292-303.

Kang J.Y., Chung C.W., Sung J.H., Park B.S., Choi J.Y., Lee S.J., Choi B.C., Shim C.K., Chung S.J., Kim D.D. (2009) Novel porous matrix of hyaluronic acid for the three-dimensional culture of chondrocytes. Int. J. Pharm. 369: 114-120.

Kisiday J., Jin M., Kurz B., Hung H., Semino C., Zhang S., Grodzinsky A.J. (2002) Self-assembling peptide hydrogel fosters chondrocyte extracellular matrix production and cell division. Implications for cartilage tissue repair. Proc. Natl. Acad. Sci. USA 99: 9996-10001.

Kisiday J.D., Jin M.S., DiMicco M.A., Kurz B., Grodzinsky A.J. (2004) Effects of dynamic compressive loading on chondrocyte biosynthesis in self-assembling peptide scaffolds. J. Biomech. 37: 595-604.

Klein-Nulend J., Louwerse R.T., Heyligers I.C., Wuisman P., Semeins C.M., Goei S.W.,. Burger E.H (1998) Osteogenic protein (OP-1, BMP-7) stimulates cartilage differentiation of human and goat perichondrium tissue in vitro. J. Biomed. Mater. Res. 40: 614-620.

Knutsen G., Engebretsen L., Ludvigsen T.C., Drogset J.O., Grontvedt T., Solheim E., Strand T., Roberts S., Isaksen V., Johansen C. (2004) Autologous chondrocyte implantation compared with microfracture in the knee. A randomized trial. J. Bone Joint Surg. Am. 86A: 455-464.

Kreuz P.C., Steinwachs M.R., Erggelet C., Krause S.J., Konrad G., Uhl M., Sudkamp N. (2006) Results after microfracture of full-thickness chondral defects in different compartments in the knee. Osteoarthr. Cartil. 14: 1119-1125.

Li Z., Kupcsik L., Yao S.J., Alini M., Stoddart M.J. (2009) Chondrogenesis of human bone marrow mesenchymal stem cells in fibrin-polyurethane composites. Tissue Eng. Part A 15: 1729-1737.

Loreto C., Lo Castro E., Musumeci G., Loreto F., Rapisarda G., Rezzani R., Castorina S., Leonardi R., Rusu M.C. (2012) Aquaporin 1 expression in human temporomandibular disc. Acta Histochem. 114: 744-748.

Loreto C., Musumeci G., Castorina A., Martinez G. (2011) Degenerative disc disease of herniated intervertebral discs is associated with extracellular matrix remodeling, vimentin-positive cells and cell death. Ann. Anat. 193: 156-162.

Marcacci M., Berruto M., Brocchetta D., Delcogliano A., Ghinelli D., Gobbi A., Kon E., Pederzini L., Rosa D., Sacchetti G.L., Stefani G., Zanasi S. (2005) Articular cartilage engineering with Hyalograft ® C - 3-year clinical results. Clin. Orthop. Relat. Res. 435: 96-105.

Marlovits S., Striessnig G., Kutscha-Lissberg F., Resinger C., Aldrian S.M., Vecsei V., Trattnig S. (2005) Early postoperative adherence of matrix-induced autologous chondrocyte implantation for the treatment of full-thickness cartilage defects of the femoral condyle. Knee Surg. Sports Traumatol. Arthrosc. 13: 451-457.

Martin J.A., Buckwalter J.A. (2003) The role of chondrocyte senescence in the pathogenesis of osteoarthritis and in limiting cartilage repair. J. Bone Joint Surg. Am. 85A: 106-110.

Miniaci A., Tytherleigh-Strong G. (2007) Fixation of unstable osteochondritis dissecans lesions of the knee using arthroscopic autogenous osteochondral grafting (Mosaicplasty). Arthroscopy 23: 845-851.

Mishra A., Tummala P., King A., Lee B., Kraus M., Tse V., Jacobs C.R. (2009) Buffered platelet-rich plasma enhances mesenchymal stem cell proliferation and chondrogenic differentiation. Tissue Eng. Part C-Methods 15: 431-435.

Mithoefer K., Williams R.J., Warren R.F., Potter H.G., Spock C.R., Jones E.C., Wickiewicz T.L., Marx R.G. (2005) The microfracture technique for the treatment of articular cartilage lesions in the knee. A prospective cohort study. J. Bone Joint Surg. Am. 87A: 1911-1920.

Mithoefer K., Williams R.J., Warren R.F., Wickiewicz T.L., Marx R.G. (2006) High-impact athletics after knee articular cartilage repair. A prospective evaluation of the microfracture technique. Am. J. Sports Med. 34: 1413-1418.

Miyanishi K., Trindade M.C.D., Lindsey D.P., Beaupre G.S., Carter D.R., Goodman S.B., Schurman D.J., Smith R.L. (2006) Effects of hydrostatic pressure and transforming growth factor-beta 3 on adult human mesenchymal stem cell chondrogenesis in vitro. Tissue Eng. 12: 1419-1428.

Mussa R., Hans M.G., Enlow D., Goldberg J. (1999) Condylar cartilage response to continuous passive motion in adult guinea pigs. A pilot study. Am. J. Orthod. Dentofacial. Orthop. 115: 360-367.

Musumeci G., Leonardi R., Carnazza M.L., Cardile V., Pichler K., Weinberg A.M., Loreto C. (2013a) Aquaporin 1 (AQP1) expression in experimentally induced osteoarthritic knee menisci: An in vivo and in vitro study. Tissue Cell. 45: 145-152.

Musumeci G., Loreto C., Carnazza M.L., Cardile V., Leonardi R. (2013b) Acute injury affects lubricin expression in knee menisci: An immunohistochemical study. Ann Anat. 195: 151-158. doi:10.1016/j.aanat.2012.07.010.

Musumeci G., Carnazza M.L., Leonardi R., Loreto C. (2012) Expression of beta-defensin-4 in "an in vivo and ex vivo model" of human osteoarthritic knee meniscus. Knee Surg. Sports Traumatol. Arthrosc. 20: 216-222.

Musumeci G., Loreto C., Leonardi R., Castorina S., Giunta S., Carnazza M.L., Trovato F.M., Pichler K., Weinberg A.M. (2013c) The effects of physical activity on apoptosis and lubricin expression in articular cartilage in rats with glucocorticoid-induced osteoporosis. J. Bone Miner. Metab. 31: 274-284.

Musumeci G., Loreto C., Clementi G., Fiore C.E., Martinez G. (2011a) An in vivo experimental study on osteopenia in diabetic rats. Acta Histochem. 113: 619-625.

Musumeci G., Loreto C., Carnazza M.L., Martinez G. (2011b) Characterization of apoptosis in articular cartilage derived from the knee joints of patients with osteoarthritis. Knee Surg. Sports Traumatol. Arthrosc. 19: 307-313.

Negrin L., Kutscha-Lissberg F., Gartlehner G., Vecsei V. (2012) Clinical outcome after microfracture of the knee: a meta-analysis of before/after-data of controlled studies. Int. Orthop. 36: 43-50.

Nho S.J., Foo L.F., Green D.M., Shindle M.K., Warren R.F., Wickiewicz T.L., Potter H.G., Williams R.J. (2008) Magnetic resonance imaging and clinical evaluation of patellar resurfacing with press-fit osteochondral autograft plugs. Am. J. Sports Med. 36: 1101-1109.

Nixon A.J., Haupt J.L., Frisbie D.D., Morisset S.S., McIlwraith C.W., Robbins P.D., Evans C.H., Ghivizzani S. (2005) Gene-mediated restoration of cartilage matrix by combination insulin-like growth factor-I/interleukin-1 receptor antagonist therapy. Gene Ther. 12: 177-186.

Ozturk A., Ozdemir M.R., Ozkan Y. (2006) Osteochondral autografting (mosaicplasty) in grade IV cartilage defects in the knee joint: 2- to 7-year results. Int. Orthop. 30: 200-204.

Pearsall A.W., Tucker J.A., Hester R.B., Heitman R.J. (2004) Chondrocyte viability in refrigerated osteochondral allografts used for transplantation within the knee. Am. J. Sports Med. 32: 125-131.

Peretti G.M., Randolph M.A., Villa M.T., Buragas M.S., Yaremchuk M.J. (2000) Cell-based tissue-engineered allogeneic implant for cartilage repair. Tissue Eng. 6: 567-576.

Peterson L., Minas T., Brittberg M., Lindahl A. (2003) Treatment of osteochondritis dissecans of the knee with autologous chondrocyte transplantation. Results at two to ten years. J. Bone Joint Surg. Am. 85A: 17-24.

Pichler K., Loreto C., Leonardi R., Reuber T., Weinberg A.M., Musumeci G. (2013) In rat with glucocorticoid-induced osteoporosis, RANKL is downregulated in bone cells by physical activity (treadmill and vibration stimulation training). Histol Histopathol. 28: 1185-1196.

Ryan J.A., Eisner E.A., DuRaine G., You Z.B., Reddi A.H. (2009) Mechanical compression of articular cartilage induces chondrocyte proliferation and inhibits proteoglycan synthesis by activation of the ERK pathway: implications for tissue engineering and regenerative medicine. J. Tissue Eng. Regen. Med. 3: 107-116.

Schachar N.S., Novak K., Hurtig M., Muldrew K., McPherson R., Wohl G., Zernicke R.F., McGann L.E. (1999) Transplantation of cryopreserved osteochondral dowel allografts for repair of focal articular defects in an ovine model. J. Orthop. Res. 17: 909-919.

Smith G.D., Taylor J., Almqvist K.F., Erggelet C., Knutsen G., Portabella M.G., Smith T., Richardson J.B. (2005) Arthroscopic assessment of cartilage repair: A validation study of 2 scoring systems. Arthroscopy 21: 1462-1467.

Steadman J.R., Briggs K.K., Rodrigo J.J., Kocher M.S., Gill T.J., Rodkey W.G. (2003) Outcomes of microfracture for traumatic chondral defects of the knee: Average 11-year follow-up. Arthroscopy 19: 477-484.

Steadman J.R., Ramappa A.J., Maxwell R.B., Briggs K.K. (2007) An arthroscopic treatment regimen for osteoarthritis of the knee. Arthroscopy 23: 948-955.

Steadman J.R., Rodkey W.G., Rodrigo J.J. (2001) Microfracture: Surgical technique and rehabilitation to treat chondral defects. Clin. Orthop. Relat. Res. 391: S362-S369.

Strauss E., Schachter A., Frenkel S., Rosen J. (2009) The efficacy of intra-articular hyaluronan injection after the microfracture technique for the treatment of articular cartilage lesions. Am. J. Sports Med. 37: 720-726.

Tran-Khanh N., Hoemann C.D., McKee M.D., Henderson J.E., Buschmann M.D. (2005) Aged bovine chondrocytes display a diminished capacity to produce a collagen-rich, mechanically functional cartilage extracellular matrix. J. Orthop. Res. 23: 1354-1362.

Trattnig S., Ba-Ssalamah A., Pinker K., Plank C., Vecsei V., Marlovits S. (2005) Matrix-based autologous chondrocyte implantation for cartilage repair: noninvasive monitoring by high-resolution magnetic resonance imaging. Magn. Reson. Imaging 23: 779-787.

van Ooij A., Oner F.C., Verbout A.J. (2003) Complications of artificial disc replacement. A report of 27 patients with the SB Charite disc. J. Spinal Disord. Tech. 16: 369-383.

Widuchowski W., Lukasik P., Kwiatkowski G., Faltus R., Szyluk K., Widuchowski J., Koczy B. (2008) Isolated full thickness chondral injuries. Prevalance and outcome of treatment. A retrospective study of 5233 knee arthroscopies. Acta Chir. Orthop. Traumatol. Cech. 75: 382-386.

Wood J.J., Malek M.A., Frassica F.J., Power J.A., Mohan A.K., Bloom E.T., Braun M.M., Cote T.R. (2006) Autologous cultured chondrocytes: Adverse events reported to the united states food and drug administration. J. Bone Joint Surg. Am. 88A: 503-507.

Wright R.W. (2009) Knee Injury Outcomes Measures. J. Am. Acad. Orthop. Surg. 17: 31-39.

Zaslav K., Cole B., Brewster R., DeBerardino T., Farr J., Fowler P., Nissen C. (2009) A prospective study of autologous chondrocyte implantation in patients with failed prior treatment for articular cartilage defect of the knee. Results of the Study of the Treatment of Articular Repair (STAR) clinical trial. Am. J. Sports Med. 37: 42-55.

Zheng M.H., Willers C., Kirilak L., Yates P., Xu J.K., Wood D., Shimmin A. (2007) Matrix-induced autologous chondrocyte implantation (MACI®): Biological and histological assessment. Tissue Eng. 13: 737-746.